PREVALENCE, ASSOCIATED RISK FACTORS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF THERMOPHILIC *CAMPYLOBACTER* SPP. OF OVINE CARCASS AT ADDIS ABABA ABATTOIR ENTERPRISE, ETHIOPIA

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LIST OF ABBREVIATIONS

AAAE	Addis Ababa Abattoir Enterprise
AIDS	Acquired Immune Deficiency Syndrome
BoD	Burden of Disease
C. coli	*Campylobacter coli*
C. jejuni	*Campylobacter jejuni*
CDC	Centre for Disease Control
CLSI	Clinical and Laboratory Standard Institute
CSA	Central Statistical Agency
DALY	Disability Adjusted Life Year
EFSA	European Food Safety Authority
FDA	Food and Drug Adminstration
FSAI	Food Safety Authority of Ireland
GBS	Guillain–Barre Syndrome
HACCP	Hazard Analysis and Critical Control Points
HIV	Human Immuno Deficiency Virus
HS	Heat-Stable (antigine)
IBS	Irritable Bowel Syndrome

LMIC Low- and Middle-Income Countries

MFS Miller Fisher Syndrome

NSM National Standard Methods

PCR Polymerase Chain Reaction

Spp. Species

WHO World Health Organization

ANNEXES

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ABSTRACT

The food borne-thermophilic *Campylobacter* species are considered to be the leading cause of human gastroenteritis worldwide with emerging antimicrobial resistant strains. Consumption of raw or under cooked meat being an important source for zoonotic infection; poultry play the major role followed by other food animals especially sheep which is a widely consumed protein source for the pubic, Ethiopia. A cross sectional abattoir based study was conducted on sheep and the carcass destined for slaughter at Addis Ababa Abattoir Enterprise. To determine the prevalence of thermophilic *Campylobacter* spp. 160 carcass and 160 rectal swabs were bacteriologically examined where 21(13.1%) and 12(7.5%) thermophilic *Campylobacter* spp. were isolated respectively. Biochemical test results of the carcass isolates indicated 12 (57.1%) to be C. *jejuni,* 6(28.6%) C. *coli* and 3(14.3%) C. *lari.* Similar examination of abattoir environment pool samples of 8 sampling days revealed 7(87.5%) to be positive for the thermophilic Campylobacters. None of wash water samples were positive for the bacteria. Antimicrobial susceptibility pattern test towards twelve antimicrobials using standard disk diffusion method resulted higher resistance percentage (42.1%) for amixacillin-clavulanic acid and (42.1%) kanamycin followed by sterptomycine, oxytetracyline and compound sulphonamide (33.3%) each. In the present study, most isolates were susceptible to ceftriaxone and clindamycin with (4.8%) resistance percentage each, and a lesser degree to erythromycin (9.5%). Multi drug resistance is observed in 52.4% of the isolates examined. Concluding, raw mutton is a potential source of campylobacteriosis with varied antimicrobial resistant strains for the public hence hygienic meat production in abattoirs and wise use of veterinary drugs are inevitable to render the public health.

Keywords: Antimicrobial resistance, sheep, swabs, thermophilic *Campylobacters.*

1. INTRODUCTION

Campylobacter is one of the major pathogens involved in food-borne illnesses with an estimated 400 million cases per year worldwide and it has been reported that only 500 cells of *C. jejuni* can cause human illness (Mpalang *et al.,* 2014; Nawal, 2011). In many countries, the organism is Campylobacteriosis in humans is characterized by watery or bloody diarrhea, abdominal cramps and nausea (Blaser, 2000; Nachamkin, 1999). An acute infection can have serious long-term consequences, including the peripheral neuropathies, Guillain–Barre syndrome (GBS) and Miller Fisher syndrome (MFS), and functional bowel diseases, such as irritable bowel syndrome (IBS) (CDC 2008; Hughes and Cornblath, 2005).

The *Campylobacter* bacterial genera contain several species of both public and animal health. Among them *Campylobacter jejuni* and *C. coli* are the most common cause of gastroenteritis in humans CDC (2008) being isolated 3–4 times more frequently from patients with alimentary tract infections than other bacterial enteropathogens (such as *Salmonella* or Escherichia *coli*) (FDA, 2012; Skirrow, 2002 and WHO, 2013). Children, the elderly and those with weakened immune system (including cancer, HIV/AIDS and transplant patients) being the risk group. Hence, the high incidence of *Campylobacter* spp. diarrhea as well as its duration and possible squeals, makes campylobacteriosis very important from a public health perspective with significant socio-economic impact (EFSA, 2010).

Campylobacter spp. are normally carried in the intestinal tracts of many domestic livestock such as poultry, cattle, sheep, pigs, as well as wild animals and birds (Mpalang *et al.,* 2014; Pezzotti *et al.,* 2003). Transmission can occur through direct contact with infected animals or from equipment, water or during carcass dressing in a slaughter line (Doyle and Beuchat, 2007). *Campylobacter* contaminated foods as the result of poor sanitation are an important potential source of infection in humans. Food-acquired campylobacteriosis accounts for up

to 74 to 85% of total cases, with poultry being the number one contributing vehicle (Andrew *et al.*, 2013). Moreover, reports from different countries indicated perevalance rate on sheep, from USA (26.4%) USDA (2014), Turkey 24.6% Ekin *et al.(*2006) and Ethiopia 38% Kassa, *et al*. (2005). Also sheep carcasses were found to be more highly contaminated with *Campylobacter* spp. than goat carcasses with rates of 10.6% and 9.4%, respectively (Woldemariam et al., 2009) amd Dadi and Asrat (2008) also reported *Campylobacter* species isolated from sheep and goat carcasses with rates of 10.5% and 7.6%, respectively.

Furthermore, *Campylobacter* with resistance to antimicrobial agents has been implicated worldwide (USDA, 2014; Nawal, 2011; Ekin *et al*., 2006 ; Kassa, *et al*. (2005)). The use of antimicrobial agents in food animals has resulted in the emergence and dissemination of antimicrobial-resistant bacteria including antimicrobial-resistant *Campylobacter*, which has potentially serious impact on food safety in both animal and human health. The situation seems to deteriorate more rapidly in the developing countries where there is a widespread and uncontrolled use of the antibiotics (Ebrahim *et al.,* 2010). Though scarce, data from low- and middle-income countries suggest that the burden of disease due to *Campylobacter* infection is considerable (WHO, 2013). In Ethiopia likewise, a few publications have reported on the occurrence and susceptibility testing of *Campylobacte*r strains to antimicrobials on human (Asrat, 2008), Asrat, *et al.* (1999); Gedlu and Assefa (1996), food animals, Kassa, *et al.* (2005) and foods of animal origin, Dadi and Asrat (2008) abattoir based, Woldemariam, *et al.*(2009) and antimicrobial susceptibility pattern on sheep carcasses Yeshimebet *et al.* (2013). And none is accessed on the status of abattoir environment contamination of operations by *Campylobacter* spp.

Therefore, the purpose of this study is to determine the prevalence, antimicrobial susceptibility pattern of thermophilic *Campylobacter* spp. from sheep carcasses and asses associated risks posed by the abattoir environment during the study period.

2. LITERATURE REVIEW

2.1. *Campylobacte*r: morphology and bacterial characteristics

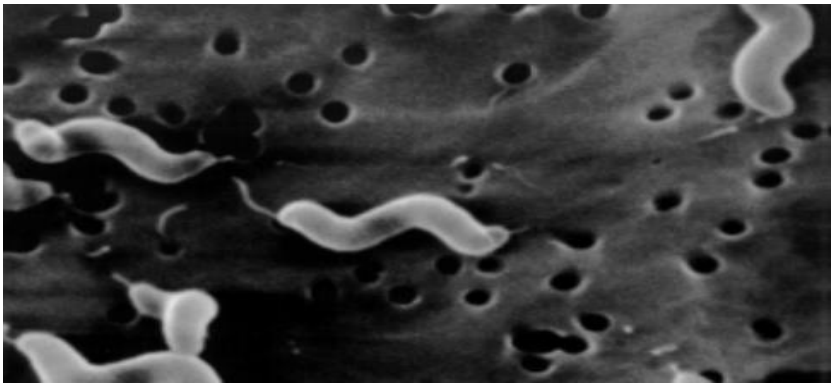

Figure 1: Scanning electron micrograph of the single polar flagellum and corkscrew shape of *Campylobacter jejuni.*

Source: Sean (1999).

2.1.1. Description of the organism

The name *Campylobacter* is derived from the Greece ‘campylos’ meaning ‘curved’ and ‘baktron ‘ meaning ‘rod’ (Blaser, 2000). *Campylobacter* spp. are Gram-negative, non-spore forming bacteria and are members of the family Campylobacteraceae. The genus *Campylobacter* comprises of 17 species and 6 subspecies (Silva *et al.,* 2011). The continual progress and developments in the criterion of taxonomy may refine the number of *Campylobacter* species. The two species most commonly associated with human disease are *C. jejuni and C. coli*. *C. jejuni* accounts for more than 80% of *Campylobacter*-related human illness, with *C. coli* accounting for up to 18.6% of human illness. *C. fetus* has also been associated with foodborne disease in humans (FDA, 2012).

2.1.2. Growth and survival characteristics

Campylobacter jejuni is adapted to the intestinal tract of warm-blooded animals. Survival of *C. jejuni* outside the gut is poor, and replication does not occur readily outside this environmental niche (Murphy *et al.*, 2006). *Campylobacter* spp. are fragile organisms. They are sensitive to freezing, heating (pasteurization/cooking), drying, acidic conditions (pickling), salinity, disinfectants and irradiation. They survive poorly at room temperature (21°C) and in general survive better at cooling temperatures (Park, 2002; Andrew *et al.*, 2013). *C. jejuni* grows best at 37°C to 42°C, in a low oxygen or microaerophilic environment, such as an atmosphere of 5% O_2, 10% CO_2, and 85% N_2 (Murphy *et al,*. 2006). Requirements for growth in the laboratory also reflect this narrow ecologic niche (Sean and Linda, 2003).

Adaptations to an intestinal niche include a single polar flagellum and corkscrew shape (Fig.1). These traits facilitate motility in the viscous intestinal mucous (Sean and Linda, 2003). *Campylobacter* spp. have been shown to enter a viable but non-culturable state when subjected to unfavourable conditions, such as low nutrient availability, elevated temperature, freezing or stationary phase (Levin, 2007). In this state, cells transform from a motile spiral form to a coccoid form (Rollins and Colwell, 1986). The nature and role of this coccoid form is uncertain. *C. jejuni* is able to adapt to aerobic conditions due to an ability to produce biofilms (Reuter *et al.*, 2010).

2.1.3. Virulence and infectivity

Campylobacter spp. have four main virulence properties: motility, adherence, invasion and toxin production. The exact nature of how *Campylobacter* spp. adhere to and invade the intestinal epithelial cells is not fully understood (Levin, 2007). It is thought that the combination of its spiral shape and flagella leads to rapid motility that enables the organisms to penetrate through the intestinal lining unlike conventional bacteria (Bhavasar and Kapadnis, 2007; Levin, 2007).

Campylobacter organisms produce two types of toxins: enterotoxin and cytotoxins. The enterotoxin of *C. jejuni* is similar to the *Vibrio cholerae* toxin and the *Escherichia coli* heatliable toxin. This enterotoxin is produced to a lesser degree by *C. coli*. It has been suggested that enterotoxin produced by *Campylobacter* spp. results in watery diarrhoea, as opposed to bloody diarrhoea due to cytotoxin production (Pickett *et al.,* 1996).

Rates of infection increased with the ingested dose, and rates of illness appeared to increase when inocula were ingested in a suspension buffered to reduce the acidity of the stomach (Bungay *et al.,* 2005). In human, it has been estimated that consumption of a small number of organisms (500 or less) may be associated with illness. Therefore, the fact that the organism does not multiply very effectively in most foods does not prevent it from causing foodborne illness (Sean and Linda, 2003; Andrew *et al.,* 2013).

2.1.4. Source of infection and transmission

The principal route by which *Campylobacter* contaminates the food is through fecal contamination by *Campylobacter* infected carriers. Mostly human campylobacteriosis are associated with handling of raw poultry, undercooked contaminated meat, cross contamination of raw and cooked foods and poor hygiene (Suzuki and Yamamoto, 2009). Raw meats and poultry become contaminated during processing when intestinal contents contact the meat surfaces. Feco-oral person to person transmission of infection has been reported for *C. jejuni.* This uncommon type of transmission can occur when personal hygiene is poor. Humans act as vectors transferring the organism into poultry production area with contaminated clothing and foot wear (Doyle and Beuchat, 2007). It is often difficult to trace sources of exposure to *Campylobacter* because of the sporadic nature of the infection, and the important role of cross-contamination (WHO, 2013).

2.2. Clinical features of campylobacteriosis

2.2.1. In humans

The clinical feature of *Campylobacter* enteritis in humans caused by *C. jejuni* and *C. coli* are indistinguishable from each other and from acute bacterial diarrhea caused by other pathogens like *Salmonella enteritis* (Skirrow, 2002). *Campylobacter* may cause mild or severe diarrhea, bloody diarrhea, nausea, and stomach pain, often with fever (Andrew *et al.*, 2013).

Abdominal pain can persist for up to 7 days and recurrence of symptoms can occur. The illness may start with cramping abdomen, diarrhea, fever, chills, headache, myalgia and occasionally delirium, with typical more intense long lasting abdominal pain and occasionally blood or mucous in the stool (Doyle and Beuchat, 2007). Extra-intestinal infection and chronic sequelae of infection occur in smaller proportion of patients (Nachamkin, 1999). Bacteremia has been noted in less than 1% of patients with *C. jejuni* infection. Meningitis and endocarditis are rare manifestation of *C. jejuni* infection. There have been infrequent reports of *C. jejuni* infections manifested as septic abortion, acute cholecystitis, pancreatitis, and cystitis (Nachamkin, 1999).

Campylobacters have also been linked to some autoimmune diseases such as Reactive Arthritis (RA) and Guillain-Barrè Syndrome (GBS). These two major late onset complications of *Campylobacter* are estimated at one case per 2000 infections (Altekruse *et al.*, 1999; WHO, 2013). *Campylobacter* infection is recognized as the most commonly identified antecedent event in GBS (40-60% of all cases), also known as post-infective polyneuropathy. The main lesions are acute inflammatory demyelinating polyradiculo-neuropathy that results in a flaccid paralysis (Weinberg *et al.*, 2001). Reactive arthritis occurs in approximately 1% of patients with *Campylobacter* enteritis (Skirrow, 2002).

2.2.2. In food or farm animals

Campylobacter spp. reside in the gut of domesticated warm-blooded animals and birds as part of the intestinal microbiota (Senok and Botta, 2009). *Campylobacter* species cause enteritis, abortions, and infertility in various species of animals. The role of *C. jejuni* as primary pathogen in farm animals is uncertain (Padungton and Kaneene, 2003). *C. jejuni* and occasionally *C. coli* cause enteritis in dogs, cats, calves, sheep, mink, poultry and some species of laboratory animals. The clinical signs may be more severe in young animals. Calves typically have a thick, mucoid diarrhea with occasional flecks of blood, either with or without fever. *C. fetus* subsp. fetus and *C. jejuni* can cause enzootic abortion that can result in late term abortions, stillbirths, and weak lambs in sheep. Infections in sheep are sometimes followed by endometritis and occasionally deaths. Morbidity may be up to 90% in outbreaks in sheep but is usually around 5 to 50%. Morbidity in sheep can result in prolonged lambing, and reduction in milk output. Recovery with immunity to re-infection is typical. Sheep can become persistently infected and continue to shed bacteria in the feces (Aiello and Mays, 1998).

2.2.3. Laboratory diagnosis

Campylobacter is difficult to isolate, grow and identify (WHO, 2013). Conventional diagnostic methods require that suspected stool specimens, feces or food samples of animals, with favorable transport and storage conditions including use of transport media in the pre-analytical phase, are cultured on selective agar at 42^{o}C under microaerophilic conditions for up to 72 hours before a negative report is issued (Senok and Botta, 2009). Only culture plates with colonies showing the characteristic *Campylobacter* morphology and oxidase positivity are then reported as *Campylobacter* spp. recognition of colonies as *C. jejuni* that are gray/moist flat, glossy, effuse colony with a tendency to spread along the inoculation track having well-spaced colonies resembling droplets of fluid and on moist agar a thin, spreading film and with continued incubation colonies become convex often with a dull surface (NSM, 2007; Hadush and Pal, 2013).

However, further identification to the species level requires other tests including growth temperature preferences, antibiotic sensitivity to cephalothin and nalidixic acid, and biochemical tests, mainly the hippurate test (Senok and Botta, 2009).

The first report on the application of polymerase chain reaction (PCR) in the diagnosis of *Campylobacter* was described by Oyofo in 1992. Application of multiplex PCR for the detection and speciation of this pathogen; however, these protocols have been optimized for isolates obtained from pure cultures and artificially spiked stool specimens (Aquino *et al.,* 2002; Persson and Olsen, 2005).

2.2.4. Treatment and antibiotic resistance

Most cases of *Campylobacter* enteritis are self-limiting, symptomatic treatment of campylobacteriosis with rehydration solutions is recommended in affected children but is of questionable benefit in otherwise healthy adults with adequate fluid intake (Anne, 2012).

In situations where antibiotic therapy is indicated either erythromycin or ciprofloxacin are the usual drugs of choice. However, recent data indicates an upward trend of *Campylobacter* resistance to antibiotics with varying patterns being seen in different countries and regions (Moore *et al.,* 2005). In addition, there is growing concern that the widespread use of antibiotics such as erythromycin, ciprofloxacin, and tetracycline in veterinary medical practice and as additives to animal feeds (particularly poultry) can select for resistant *Campylobacter* spp. which may be transmitted to humans through the food chain (Pezzotti *et al.,* 2003; Moore *et al.,* 2005).

2.3. Public health significance of *Campylobacter*

According to the Centre for Disease Control (CDC) report, *Campylobacter* infections accounted for approximately one-third of laboratory confirmed food borne illness that occurred globally in food net surveillance areas (CDC, 2008).

2.3.1. Reported incidence of campylobacteriosis

The true incidence of gastroenteritis due to *Campylobacter* spp. is poorly known, particularly in LMIC; studies in high-income countries have estimated the annual incidence at between 4.4 and 9.3 per 1000 population (WHO, 2013). Generally, developing countries do not have national surveillance programs for campylobacteriosis; therefore, incidence values in terms of number of cases for a population do not exist. Most estimates of incidence in developing countries are from laboratory-based surveillance of pathogens responsible for diarrhea. *Campylobacter* isolation rates in developing countries range from 5 to 20% (Table 1) (Oberhelman and Taylor, 2000).

Table 1: Isolation rates of *Campylobacter* from diarrhea specimens from <5-year-olds in selected developing countries.

WHO region and country	Isolation rate (%)
Africa	
Algeria	17.7
Cameroon	7.7
Ethiopia	13.8
Nigeria	16.5
Tanzania	18.0
Zimbabwe	9.3
Americas	
Brazil	9.9
Guatemala	12.1

Source: Akitoye *et al.* (2002).

2.3.2. Food born implications of Campylobacter

Food-acquired campylobacteriosis accounts for up to 74 to 85% of total cases, with poultry being the number one contributing vehicle (Andrew *et al.*, 2013). Campylobacter-contaminated foods the result of poor sanitation are an important potential source of infection in humans (Table 2 and 3). For example, campylobacters were isolated from 40% and 77% of retail poultry meat sold in Bangkok, Thailand, and Nairobi, Kenya, respectively (Akitoye *et al.*, 2002). The serotypes of the organisms isolated in Thailand were similar to those of organisms isolated from humans. In Mexico City, a survey of ready-to-eat roasted chickens showed that they were contaminated with campylobacters (Quinones-Ramirez, 2000). In developed countries, risk factors associated with foods include occupational

exposure to farm animals, consumption of raw milk or milk products, and unhygienic food preparation practices (Akitoye *et al.,* 2002).

Table 2: Selected major foodborne outbreaks associated with *Campylobacter* spp. (>50 cases and/or ≥1 fatality).

Year	No. of Cases (fatalities)	Food	Country
2008	98	Raw peas	US
2007	68	Cheese	US
2005	79	Chicken salad	Denmark
2005	86	Chicken liver pate	Scotland
2003	81	Custard prepared from UHT milk	Spain
1998	79	Tuna salad	US
1995	78	Cucumber	South Australia

Source: Anne (2012).

Table 3: Prevalence of *Campylobacter* in food of animal source, Addis Ababa.

Sample type	Abattoir	Butchers'shop	Supermarket	Total
Beef	9/138 (6.5)	4/69 (5.8)	1/20 (5.0)	14/227(6.2)
Mutton	11/93 (11.8)	1/10 (10.0)	0/11 (0)	12/114 (10.5
Goat	6/67 (9.0)	1/11 (9.0)	0/14 (0)	7/92 (7.6)
Pork	3/30 (10.0)	-	1/17 (5.9)	4/47 (8.5)
Chicken	8/30 (26.7)	-	5/30 (16.7)	13/60 (21.7)
Total	37/358	6/90(6.7)	7/92 (7.6)	50/540 (9.3)

Source: Dadi and Asrat (2008).

2.3.3. Estimates of impact of human campylobacteriosis in developing countries

The Disability Adjusted Life Year (DALY) is the basic unit used in Burden of Disease (BoD) methodology to quantify the impact of disease on a population. DALYs have been applied in the Dutch population to measure the mean health burden of Campylobacter-associated illness in the period 1990–1995. The mean estimate was 1,400 DALYs per year; the main determinants of health burden were acute gastroenteritis (440 DALYs), gastroenteritis-related mortality (310 DALYs) and residual symptoms of GBS (340 DALYs) (Mangen *et al.,* 2004).

Although data on DALYs due to campylobacteriosis in developing countries are not available, diarrhea, which is a clinical manifestation of campylobacteriosis, was one of the top three causes of death and disease in developing countries in 1990. The disease is projected globally to remain one of the top 10 by 2020. (The burden of campylobacteriosis in developing countries may increase by 2020 because HIV is projected to move up to the 10^{th} position from 28^{th} by 2020). Considering the higher incidence of campylobacteriosis in developing countries, DALYs for the disease in developing countries will likely be higher than those of the Dutch population (Akitoye *et al.,* 2002).

2.3.4. Factors influencing campylobacteriosis epidemiology

Age

Campylobacteriosis is often a pediatric disease especially in developing countries. This is because of multiple reasons; as age increases, level of antibody tends to increase. Higher risk of campylobacteriosis in young children was also associated with ownership of pet chickens (Sean and Linda, 2003).

Season

In developed countries epidemics occur in summer and autumn. Isolation peaks vary from one country to another and also within countries; in contrast, in developing countries, *Campylobacter* enteritis has no seasonal preference. The lack of seasonal preference may be due to lack of extreme temperature variation as well as lack of adequate surveillance for epidemics (Akitoye *et al.*, 2002).

Travel and food trade

Foreign travel is a commonly reported risk factor for campylobacteriosis. In Sweden, where *Campylobacter* contamination of poultry meat is uncommon, international travel has traditionally accounted for approximately 75% of human *Campylobacter* infections. In the United States, it is estimated that between 20 and 25% of *Campylobacter* infections are acquired during international travel. Campylobacteriosis was the most frequently reported enteric bacterial infection in Austrian tourists returning from southern Europe and Asia. In England, travel to South Africa was associated with *C. coli* infection. The causal exposures for travel-associated infections remain to be determined (Sean and Linda, 2003; Anne, 2012).

Strain variation

Although a diverse group of strains is associated with Guillain-Barré syndrome (GBS), the syndrome is strongly linked to a few strains of *C. jejuni* (eg. heatstable or Penner serotype HS:19 and HS:41). *Campylobacter* strains contain sialic acid linkages to lipooligosaccharides resembling sialic acid moieties on the gangliosides of peripheral nerve tissues. Patients with GBS develop antibodies against these gangliosides, resulting in autoimmune targeting of peripheral nerve sites. Complement-mediated damage and blockage of neurotransmission are suspected to affect GBS pathogenesis (Sean and Linda, 2003).

Host immunity

Acquired immunity is generally accepted to be an important factor in the epidemiology of campylobacteriosis (EFSA, 2010). Prior exposure to *Campylobacter* may result in at least partial protective immunity. Since immunity may be strain specific, time-limited, and/or inadequate in the presence of large challenge doses, repeated or chronic exposure to a variety of *Campylobacter* strains may be required to produce protective immunity (Anne, 2012). In developing countries, healthy children and adults are constantly exposed to *Campylobacter* antigens in the environment. As a consequence, the levels of antibodies tend to be much higher than those in children in the developed world such as in the United States (Akitoye *et al.*, 2002).

2.3.5. Economic significance of campylobacteriosis

Campylobacteriosis cause severe economic loses both in the public health and food industry sector. Campylobacteriosis has an enormous economic impact in terms of treatment costs, loss of production, and human welfare. In livestock, particularly sheep and cattle, *Campylobacter* species are the cause of important economic losses associated with infertility problems and abortion (Beatriz and Ana, 2011).

A study, estimating the disease burden and the cost-of-illness, in Netherland indicated that cost-of-illness were direct health-care costs (e.g. doctors' consultations, hospitalization, rehabilitation), direct non-health-care costs (e.g. travel costs of patients, co-payments by patients) and indirect non-health-care costs (productivity losses), using cost estimates for a year 2000. The results, costs-of-illness were estimated to total € 21 million per year with a 90% confidence interval of between € 11 million and € 36 million per year. Concluding, *Campylobacter* infections pose an important public health problem for the Netherlands and incur substantial costs (Mangen *et al.*, 2004).

2.4. Control of the transmission of *Campylobacter* spp. In the food chain

2.4.1. Overview

The complex epidemiology of *Campylobacter*, a multi-tiered approach to control is needed, taking into consideration the different reservoirs, pathways, exposures, and risk factors (Fig.2) (WHO, 2013; Andrew *et al.*, 2013). Control of *Campylobacter* spp. throughout the food chain requires implementation of food safety management systems based on well-established principles such as those of the Hazard Analysis Critical Control Point (HACCP) system. That is a structured systematic approach to achieving food safety which involves identifying potential hazards and measures for their control. However, in the interests of control HACCP based principles should be applied by all sectors of the food industry (FSAI, 2002).

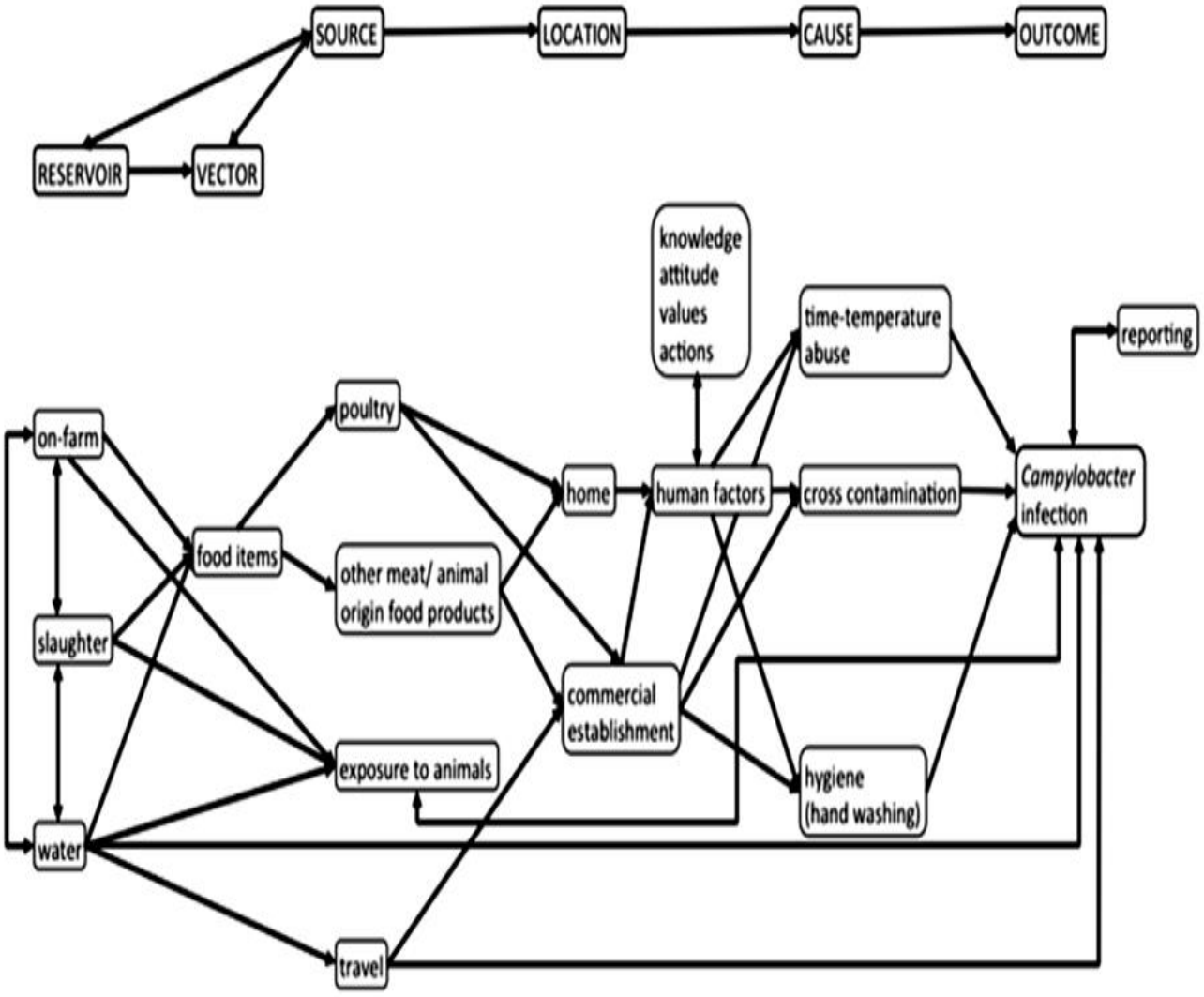

Figure 2: *Campylobacter* risk factor diagram shows the source of *Campylobacter* organisms and the locations where people are exposed.

Source: Andrew *et al.* (2013).

2.4.2. On-farm control

The interventions that have consistently been shown to be effective at pre-harvest are the application of strict biosecurity and good animal husbandry and health measures (WHO,

2013). Control of *Campylobacter* contamination on the farm may reduce contamination of carcasses, poultry, and red meat products at the retail level. Epidemiologic studies indicate that strict hygiene reduces intestinal carriage in food producing animals (Humphrey *et al.*, 1993). In field studies, poultry flocks that drank chlorinated water had lower intestinal colonization rates than poultry that drank unchlorinated water (Gregory *et al.*, 1997). Recent studies undergone to develop methods such as treatment of chickens with commensal bacteria other than *Campylobacter*, which is called competitive exclusion regimens and flock vaccination (Lis *et al.*, 2003).

2.4.3. The abattoir: the post-harvest phase

Good hygienic practices and the application of control measures based on HACCP principles are also critical for successful post-harvest control, and decontamination of the carcass by physical or chemical means (WHO, 2013). Bacterial counts on carcasses can increase during slaughter and processing steps. In one study, up to a 1,000-fold increase in bacterial counts on carcasses was reported during transportation to slaughter. Hazard Analysis Critical Control Points (HACCP) studies of the slaughter process show specific areas where contamination occurs (Andrew *et al.*, 2013).

In studies of chickens and turkeys at slaughter, bacterial counts increased by approximately 10- to 100-fold during defeathering and reached the highest level after evisceration. However, bacterial counts on carcasses decline during other slaughter and processing steps such as: Forced-air chilling of swine carcasses caused a 100-fold reduction in carcass contamination. In turkey plants, scalding reduced carcass counts to near or below detectable levels (EFSA, 2010). Adding sodium chloride or trisodium phosphate to the chiller water in the presence of an electrical current reduced *C. jejuni* contamination of chiller water by $2_{\log}10$ units. Use of chlorinated sprays and maintenance of clean working surfaces resulted in a 10- to 100-fold decrease in carcass contamination. In another study, lactic acid spraying of swine carcasses reduced counts by at least 50% to often undetectable levels (Sean *et al.*, 1999).

A radiation dose of 2.5 KGy reduced *C. jejuni* levels on retail poultry by 10 $_{\log}10$ units. However, some consumers report that the color and texture of chicken fillets are altered by irradiation. Competitive exclusion products have also been proposed to reduce broiler colonization. Various products containing defined poultry isolates of *C. jejuni,* Lactobacillus, and undefined cultures are reported to reduce colonization under experimental conditions. Diet may also alter intestinal carbohydrates that affect the colonization potential of *Campylobacters* (Sean and Linda, 2003).

2.4.4. At home

At home, the consumer is the last link in the food chain and has to deal with residual pathogens in food. The measures required in the kitchen to minimize risk of infection with *Campylobacter* spp. consist of the application of the basic principles of safe food preparation. In addition to awareness of basic measures such as hand washing and separation of ready-to-eat and raw food, some traditional food preparation practices should be discouraged. For example, the practice of washing dressed poultry carcasses in the kitchen sink is unnecessary and increases the risk of contamination (FSAI, 2002).

Proper and hygienic preparation of food, avoidance or heating of unpastuerized dairy products, avoidance of eating raw meat, travel to underdeveloped countries (hyper-endemic *Campylobacter* transmission area), and exposure to animals such as pet animal with diarrhea (particularly puppies and kittens) should be avoided (Skirrow, 2002).

2.4.5. Water

Untreated water has been identified as an important source of *Campylobacter* infections in humans. The presence of *Campylobacter* in surface water and shallow wells is likely the result of contamination by wild bird feces, manure run-off from dairy or poultry farms, or human sewage (Anne, 2012). The chlorination of carcass wash water, an important component of the HACCP programs in processing plants contributed to the decline in human campylobacteriosis (Sean and Linda, 2003). Therefore, the use of chlorinated water

in the farm as well as in abattoir or processing industries is crucial, as piped waters prevent fecal contamination from farm run offs.

2.4.6. Disease surveillance and public awareness

Surveillance of enteric diseases, including campylobacteriosis, is common in high-income countries; it is rarely attempted in other parts of the world. Nevertheless, a well-designed surveillance program for campylobacteriosis can provide information to inform national decision-making by: determining the relative importance of campylobacteriosis compared with other enteric infections; showing which animals are the primary reservoirs for infection; and helping to identify the most common pathways of transmission (WHO, 2013). Educating farmers on improved disease prevention measures and hygiene may lead to a lower prevalence of *Campylobacter* (Wagenaar *et al.*, 2006).

3. MATERIALS AND METHODS

3.1. Study Location

The study was carried out at Addis Ababa Abattoir Enterprise which is currently situated near the city center (Addis Ababa) a place locally called “kera” (Fig.3). Addis Ababa is the capital city of Federal Democratic Republic of Ethiopia and it has an area of 51 thousand hectare in the central highlands with an average altitude of 2000-3000 meters above sea level. The area is characterized by bimodal rainfall with an average of 1100 mm, the highest percentage of rain falls during the long rainy season from June to September and the short rainy season is from February to April. The average annual daily temperature ranges from 10.7°c to 23.6°C minimum and maximum, respectively and relative humidity varying from 70% to 80% during rainy season and from 40% to 50% during the dry season. Addis Ababa has an estimated human population of 3.15 million (CSA, 2007).

Addis Ababa Abattoir Enterprise (AAAE), founded in 1956 as a Share company and became a governmental organization in 1984, is the biggest abattoir in Addis Ababa serving almost all parts of the city in slaughtering and meat distribution services, 85% of the total meat consumption in Addis Ababa was covered by AAAE where the remaining 15% was from Kara- Alo Abattoir, Abattoirs from nearby oromia cities and illegal slaughtering. AAAE has its main factory in Kera and its branch in Akaki Kaliti. The enterprise has a total capacity of slaughtering 2000 cattle, 1000 sheep and goats, 100 pigs, and 10 camels per 8 hours. There were four Christian slaughtering rooms (three for cattle and one for sheep and goat), two Muslim slaughtering rooms (one for cattle and one for sheep and goat), and one pig slaughtering room in the main factory found in Kera. The enterprise has 35 vehicles for transporting meat to the customers and 6 vehicles for transporting live animals from different nearby markets to the abattoir (Gudeta, 2012).

As indicted by livestock markets and abattoirs study in Addis Ababa city, Gudeta (2012), 27% of the slaughter animals in Addis Ababa enter through Gojam Gate from different places found in North Shoa. Through the Ambo Gate, mainly from Wellega, Ginchi, Guder, and Kata, 26% of the animals were estimated to enter the city. The Dessie Gate, where Dessie, Debrebrhan, and Sheno were thought to be the main sources, was believed to be an entrance for 23% of the slaughter animals in the capital. Harar, Adama, Awash, and Arsi were estimated to be the sources for 18% of the slaughter animals entering through Akaki Gate. The other 6% of the animals in the city were assumed to enter through Jimma Gate where Jimma and Walayita areas were thought to be the main sources (Gudeta, 2012).

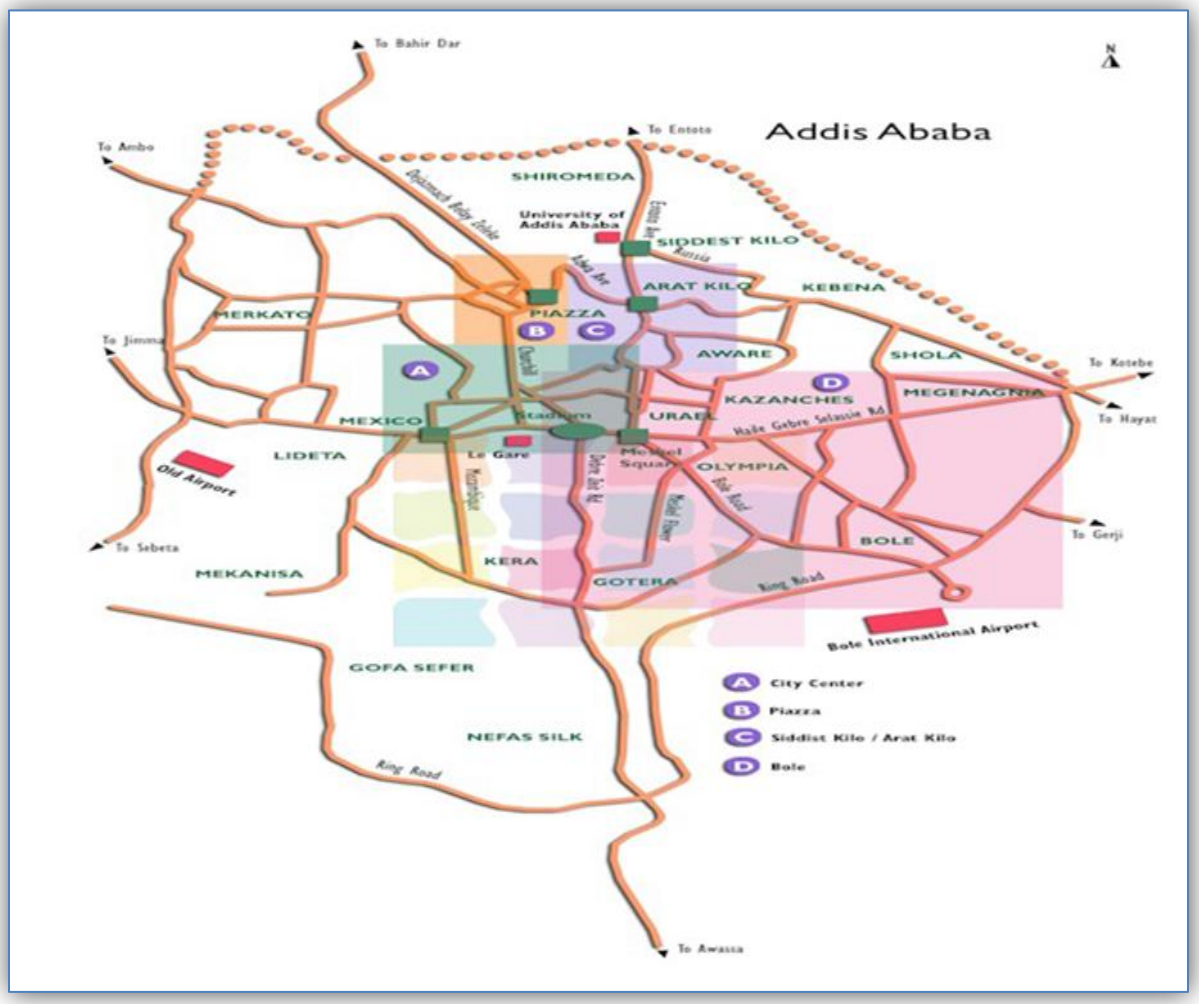

Figure 3: Map of Addis Ababa.

Source: www.addisallaround.com

3.2. Study design

A cross-sectional study was conducted from November 2013 to May 2014, to determine the prevalence of *Campylobacter jejuni* and *coli* on sheep carcasses, antibiotic susceptibility pattern of the isolates and assess associated risk factors for carcass contamination at AAAE.

3.3. Study population

Apparently healthy sheep which brought to AAAE for slaughter during study period were systematically identified and sampled for carcass and fecal swab samples. The study animals were originated from different parts of the country with different agro ecological zones, these areas include Arsi, Wellita, Wollo, Afar, Harrarge, Addis Ababa And its peripheries.

3.4. Sample size determination and sampling method

For microbiological study of *Campylobacter*, the sample size calculated based on the assumption that 11.8% expected prevalence and 5% of desired absolute precision and 95% confidence interval. Using the formula recommended by Thrusfield (2005), 160 sheep were systematic randomly selected.

$$n = \frac{1.96^2 \, Pexp \, (1-Pexp)}{d^2}$$

Where, n = required sample size, Pexp = expected prevalence, d = desired absolute precision

Accordingly, 160 rectal swabs were collected from sheep on the lairage and carcass swab samples from those160 sheep after wash were collected (2 samples from a single sampling

unit) for isolation and identification of thermophilic *Campylobacter* species during the study period. Environmental and abattoir-water samples were also collected for each sampling days (eight), environmental samples were polled swab samples from personnel hands, knives, hooks, abattoir wall, and apron. Totally, 336 swab samples were collected during the study period for isolation, identification and antimicrobial susceptibility patterns investigation of thermophilic *Campylobacter* spp. with associated risk factor assessment on slaughtering processes at Addis Ababa Abattoir Enterprise.

3.5. Sampling technique

Selected carcasses were swabbed using sterile cotton tipped swab (2X3 cm) fitted with shaft on specific sites of a carcass, the abdomen (flank), thorax (lateral), crutch, breast (lateral), which are sites with the highest rate of contamination (ISO 17604, 2005). A sterile cotton for each sites was first soaked in an approximately 10 ml of alkaline peptone water (Oxoid Ltd., Hampshire, England) rubbed first horizontally and then vertically several times on the carcasses. Swab samples from four sites of right and left side of an animal was taken as a pool. On completion of the rubbing process, the shaft was broken by pressing it against the inner wall of the test tube and disposed leaving the cotton swab in the test tube. A second dry sterile cotton swab of the same type was used as before over the entire sampled area. Swab samples were collected by use of commercially available transport tubes, containing transport medium, alkaline peptone water, that protect *Campylobacter* spp. from drying out and the toxic effects of oxygen as recommended by OIE (2008).

Rectal swab samples were obtained from each sampling unit and placed into a sterile screw capped container containing 10ml of alkaline peptone water. Separate sterile disposable gloves were used for each animal OIE (2008). Environmental samples were taken from the surfaces of walls, personnel hands, knifes, hooks, apron with sterile cotton tipped swabs on each sampling days as a pooled sample in a single screw capped test tubes containing transporting medium. The surfaces were sampled by sterile cotton wool swabs (3 cm long

and 2 cm in diameter) on wooden sticks. Each cotton wool swab was moistened with 0.1% peptone water prior to its use. The swabs were rubbed on sites continuously for 30 seconds and transferred to a sterile screw-capped test tube containing 10 ml of sterile maintenance medium (0.85% NaCl and 0.1% peptone). Washing water samples were collected similarly for each sampling days, ten ml of washing water was also collected in sterile test tube.

All samples were transport to the Microbiology Laboratory of Institute of Biodiversity being in the ice box and subsequent processing was therefore done as rapid as possible with in maximum four hour time as Campylobacters are remarkably sensitive to environmental conditions, including dehydration, atmospheric oxygen, sunlight and elevated temperature. Ice box with ice packs was used for transportation of screw capped sampling containers.

3.6. Isolation and identification of *Campylobacter* spp.

Swab samples from transport medium was streaked on to *Campylobacte*r blood free selective agar base with SR155E supplement, modified Cephoperazone Charcoal Deoxycholate Agar (mCCDA; from Oxoid Ltd.) and streaked plates were incubated at 42°C in anaerobic jar under a microaerophilic atmosphere condition (85% N2, 10% CO2, 5% O2) produced from gas generating sachets (Campy-Gen^{TM}; Oxoid Ltd.) for 48h and plates with no growth were incubated in a microaerophilic condition for additional 24h.

One presumptive *Campylobacter* colony from each selective agar plate was sub-cultured and tested by standard microbiological and biochemical procedures. In other words preliminary identification of *Campylobacter* species was performed based on microscopy to see characteristic darting motility with the iris diaphragm closed effectively to contrast the field. Gram stained morphology showed a Gram negative organism with an ‘S’shaped appearance. Positive results with oxidase, catalase tests identified thermotolerant *Campylobacter* genera. The entire above laboratory finding were recorded. The colonies of

Campylobacter from blood agar medium were picked up with a sterilized cotton swab and put into small tubes containing storage medium (Brain- heart- infusion broth medium) for identification (Annex 2).

Hippurate hydrolysis and susceptibility to nalidixic acid (30 µg) disk was evaluated and interpreted, these parameters formed the basis for the identification of *C. jejuni, C. coli* or *C. lari.* (Annex 3).

3.7. Antimicrobial susceptibility test

Antimicrobial susceptibility test for *Campylobacter* spp was performed using the standard agar disk diffusion method as recommended by Clinical and Laboratory Standards Institutions (CLSI). *Campylobacter* species were tested for the following antimicrobial agents (obtained from oxoid Ltd. UK) ampicillin (AMP) 10µg, amoxicillin with clavulanic acid (AMC) 30µg, oxytetracycline (OT) 30µg, ceftriaxone (CRO) 30µg, erythromycin (E) 15µg, clindamycin (DA) 10µg, trimethoprim (W) 5µg, Kanamycin (K) 30µg, streptomycine (S) 25µg, penicillin G (P) 10µg, compound sulphonamide (S_3) 300µg and nalidixic acid (NA) 30µg.

Three to four morphologically identical colonies of bacteria from fresh culture were picked and suspended in sterile normal saline. Turbidity of the broth culture was measured with turbido meter with in Absorbance range of 0.08 to 0.1 which is equivalent with that of 0.5 McFarland turbidity standards. A loop full of the bacterial suspension was placed at the center of Muller Hinton agar media (Oxoid, Ltd) supplemented with 5% sheep blood and evenly spread using sterile cotton tipped applicator. After drying, the above mentioned twelve antibiotic disks were placed on 120 mm petridishes and incubated at 42°C for 48 hours in anaerobic jar using CO_2 generating kits (CampyGen^{TM} Oxoid Ltd).

A standardized reference strain of *E. coli* (ATCC 25922), sensitive to all the antimicrobial drugs being tested was used as a control for the study. Finally, the diameter of the zone of inhibition around the disks was measured to the nearest millimeter using a metal caliper and

the isolates were classified as sensitive(S), intermediate (I) and resistant(R) according to the standardized table supplied by the manufacturer (CLIS, 2012). *Campylobacter* strains that were sensitive to naldixic acid considered as C. *jejuni* and C. *coli*, while strains that were resistant considered as C. *lari* (CLIS, 2005).

3.8. Data storage and analysis

All the research findings were stored in Microsoft Excel and prepared for analysis. Descriptive statistics and Chi-square test were performed using SPSS (Statistical Package for the Social Sciences) version 20 statistical software and differences were considered significant at values of $p<0.05$.

4. RESULTS

4.1. Prevalence

Sheep carcasses of 160 were investigated after wash for thermophilic *Campylobacter* species. From which, 21 (13.1%) were positive for *Campylobacter* species (Table 1). The numbers and percentages of *Campylobacter* strains isolated from sheep carcasses were 12/21 (57.1%) for C. *jejuni*, 6/21 (28.6%) for C. *coli* and 3/21(14.3%) for C. *lari.*

Table 4: Prevalence of thermophilic *Campylobacter* species in sheep carcass.

	Campylobacter spp No. (%)			
	C. jejuni	*C. coli*	*C. lari*	Total
Sheep carcass (n=160)	12(57.1)	6(28.6)	3(14.3)	21(13.1)

4.2. Isolation rates of thermophilic Campylobacters from rectal swab

Examination of 160 sheep for intestinal carriage by rectal swab revealed that 12(7.5%) were harboring thermophilic *Campylobacter* spp. Emphasizing animals (feces) are an important source of bacterial carcass contamination under unhygienic slaughtering operation procedure. The rectal isolates of sheep were not biochemically processed for species identification.

4.3. Isolation rates of thermophilic Campylobacters from associated risk factors for carcass contamination

Environmental samples - swabs of personnel hands, knives, hooks, abattoir wall and apron were taken as pooled sample for each sampling days, a total of 8 environmental samples were examined and 7(87.5%) of them were positive for thermophilic *Campylobacter* spp. results of this study do not show the specific site of contamination and there might be multi contamination taken as a single positive result. Whereas, 8 samples of wash water was examined for sampling days and no thermophilic *Campylobacter* spp. were isolated.

4.4. Antimicrobial susceptibility testing of carcass *Campylobacter* isolates

Twenty one *Campylobacter* spp. isolates from sheep carcass were subjected to antimicrobial susceptibility tests using disk diffusion method towards twelve antimicrobial agents. Interpretation of the result was based on antimicrobial break points suggested by CLSI (2012) for Enterobacteriacea since there were no recommended antimicrobial break points for *Campylobacter* spp.

The highest level of resistance of the *Campylobacter* isolates was recorded to kanamycine and amoxicillin-clavulanic acid (each 42.1%) while the least resisted antimicrobials for this specific test were ceftriaxone and clindamycin (each 4.8%). Oxytetracycline, streptomycine and compound sulphonamide (each 33.3%), nalidixic acid (23.8%), trimethoprim and penicillin G (each 19.0%), ampicilline and erythromycin (each 9.5%) being in between (Table 5).

Relative resistance of thermohpilic *Campylobacter* strains showed, the higher resistance frequencies of *C. jejuni* were observed against amoxicillin- clavulanic acid (41.7%) and kanamycin, streptomycine, oxytetracycline and compound sulphonamide (each 25.0%)

although there were significant differences when compared with those of *C. coli* ($p < 0.05$) (Table 5). Higher resistance of the C. *coli* isolates was shown to trimethoprim, kanamycin, streptomycine, penicillin G and amoxicillin-clavulanic acid (each 33.3%), followed by oxytetracycline, ampicillin, ceftriaxone, compound sulphonamide and erythromycin (16.7%) each (Table 5). *C. lari* were resistant to most of antimicrobials (100%) including for nalidixic acid, kanamycin, oxytetracycline and compound sulphonamide, (66.7%) for sterptomycine and (33.3%) for trimethoprim and amoxcilline-clavulanic acid each (Table 5).

Table 5: The antimicrobial susceptibility pattern of C. *jejuni*, C. *coli*, and C. *lari* isolated from carcasses of sheep at Addis Ababa Abattoir Enterprise.

	C. *jejuni*			C. *coli*			C. *lari*		
	S	I	R	S	I	R	S	I	R
NA	10(83.3)	-	2(16.7)	6(100)	-	-	-	-	3(100)
W	11(91.7)	-	1(8.33)	4(66.7)	-	2(33.3)	2(66.6)	-	1(33.3)
K	7(58.3)	2(16.7)	3(25.0)	3(50.0)	2(33.3)	1(16.6)	-	-	3(100)
S	7(58.3)	2(16.7)	3(25.0)	3(50.0)	1(16.6)	2(33.3)	-	1(33.3)	2(66.6)
DA	10(83.3)	1(8.33)	1(8.33)	5(83.3)	1(16.6)	-	1(33.3)	2(66.6)	-
OT	7(58.3)	2(16.7)	3(25.0)	4(66.7)	1(16.6)	1(16.6)	-	-	3(100)
P	10(83.3)	-	2(16.7)	4(66.7)	-	2(33.3)	3(100)	-	-
AMP	11(91.7)	-	1(8.3)	5(83.3)	-	1(16.6)	3(100)	-	-
AMC	11(91.7)	-	1(8.3)	5(85.3)	-	1(16.6)	3(100)	-	-
CRO	8(66.7)	4(33.3)	-	4(66.7)	1(16.6)	1(16.6)	2(66.6)	1(33.3)	-
S_3	9(75.0)	-	3(25.0)	5(83.3)	-	1(16.6)	-	-	3(100)
E	8(66.7)	3(25.0)	1(8.33)	4(66.7)	1(16.6)	1(16.6)	-	3(100)	-

NA=Nalidixic Acid, AMC=Amoxcilline- clavulanic acid, S=Streptomycine, DA=Clindamycin W=Trimethoprim, CRO=Ceftriaxone, K=Kanamycin, S_3=Compoundsulphonamide, AMC=Ampicilline, E=Erythromycin, OT=Oxytetracycline
S =SENSUTIVE I=INTERMIDIAT R=RESISTANT

Multi-resistance to two or more antimicrobials was seen in 52.4% (11/21) of *Campylobacter* strains. From these multidrug resistant strains 28.6% were *C. jejuni* followed by 14.3% *C. lari* then 9.5% *C. coli.* The multi-antimicrobial resistance for maximum numbers of antimicrobial disks (seven antimicrobials) observed was registered by *C. coli* and *C. jejuni* strain. Considering the multi-resistance proportion for each strain, six of 12 (58.3%) *C. jejuni* isolates and two of 6 (33.3%) *C. coli* isolates were resistant to two or more antimicrobials tested. All *C. lari* isolates were multi-resistant three of 3 (100%) (Table 6).

Table 6: Multi-resistant property of *Campylobacter* strains isolated from sheep carcass to two or more antimicrobial agents at Addis Ababa Abattoir Enterprise.

Combinations of drugs	All *Campy*. Strains	*C. jejuni* $n^a=12$	*C. coli* $n^b=6$	*C. lari* $n^c=3$
W-K-S-P-AMC-S3	2(9.5)	1(8.3)	1(16.7)	-
NA-K-OT-S3	1(4.8)	-	-	1(33.3)
S-AMC	1(4.8)	1(8.3)	-	-
NA-W-K-S-OT-AMC-S3	1(4.8)	-	-	1(33.3)
W-OT-P-AMP-AMC-CRO-E	1(4.8)	-	1(16.7)	-
K-S-DA-OT-S3-E	1(4.8)	1(8.3)	-	-
OT-AMC	1(4.8)	1(8.3)	-	-
OT-P-AMP-AMC-S3	1(4.8)	1(8.3)	-	-
NA-K-S-OT-S3	1(4.8)	-	-	1(33.3)
NA-K	1(4.8)	1(8.3)	-	-
Total % n = 21	11(52.4)	6(28.6)	2(9.5)	3(14.3)

n= no of carcass *Campylobacter* isolates tested for antimicrobial susceptibility.

NA=Nalidixic Acid, AMC=Amoxcilline- clavulanic acid, S=Streptomycine, DA=Clindamycin W=Trimethoprim, CRO=Ceftriaxone, K=Kanamycin, S3=Compoundsulphonamide, AMC=Ampicilline, E=Erythromycin, OT=Oxytetracycline

5. DISCUSSION

In this study, 13.1% of sheep carcasses after wash were contaminated by thermophilic *Campylobacter* spp. this finding is in agreement with a report from Iran 13.1% Ebrahim (2010). Also comparable with previous abattoir studies in Ethiopia 11.8% by Dadi and Asrat (2008) and 11.0% by Woldemariam *et al.* (2009). The slightly higher isolation rate on the present study could probably be due to the focus of the previous studies on export abattoirs which have relatively better establishment and hygienic slaughter operation. On the other hand slightly lower than a recent report from North- Shoa (21.4%) Yeshimebet *et al.* (2013) and (15.3%) Aquino *et al*. (2002). The probable justification for the above condition could be the sampling methodology used for carcass swabs on the previous studies that is, additional to carcass surface, deep swab samples were collected. Carcass swab samples for the present study were collected from four sites as a pool hence rate of specific contamination was not the study interest nevertheless previous researches on the topic (different sites of carcass) indicated no significant variation Woldemariam *et al*. (2009); Yeshimebet *et al.* (2013).

Among *Campylobacter* strains isolated in the present study from sheep carcasses *C. jejuni* was the predominant which account (57.1%) followed by *C. coli* and *C. lari* being last (28.6%) and (14.3%) respectively, this finding is in agreement with other reports Woldemariam *et al.* (2009) 59.3% *C. jejuni*, 40.7% *C. coli* and Yeshimebet *et al.* (2013) C. *jejuni* 93.3 *and C. coli* 6.7%. Similarly, a study from Eastern Turkey reported (51.9%) *C. jejuni*, (11.1%) *C.coli* and (11.1%) *C. lari* Ekin *et al.* (2006); from Iran (81.8%) *C. jejuni* and the rest (18.2%) *C. coli* Ebrahim (2010).

It has been well established that during the slaughtering process, the main sources of contamination are the slaughtered animals themselves, the staff and the work environment (Bell and Hathaway, 1996). The contamination of equipment, materials, and workers' hands can spread pathogenic bacteria to non-contaminated carcasses. This fact was strengthened by findings from this study; 7.5% of the animals examined for rectal swab

were positive which is comparable with 7.1% report from Lagos (Uaboi-Egbenni *et al.,* 2008), 6.8% reported from Nigeria, Raji *et al.* (2000) and 10.6% Yeshimebet *et al.* (2013). However, considerably lower than a report from study undergone on farm animals from western Ethiopia (38%) (Kassa *et al.,* 2005). This could be accounted by difference in agro ecological zones for the studies.

As shown on the above paragraphs the prevalence of thermophilic *Campylobacter* spp. in the carcass (13.1%) is higher than that of intestinal carriage (7.5%) this was in agreement with studies on the live animals and carcass 10.6% and 21.4% Yeshimebet *et al*. (2013). Similarly, a report from Congo isolation rates of thermophilic *Campylobacters* on goat meat and feces showed a higher isolation rate from the meat (47.8%) than the fecal sample (33.3%) (Mpalang *et al.,* 2014). These findings suggest that unhygienic slaughtering operations which lead to contaminated abattoir environment as well as carcass cross contamination are the potential risk factors for higher rate of *Campylobacter* isolation.

During the study period 87.5% of abattoir working environment was contaminated for the sampling days at random timings of the slaughter operation by this specific bacterium. Environmental contamination results from this study do not indicate the specific contaminating points of the slaughter operation since samples were taken as a pool from different sites to generally assess the persistence of the bacteria in the environment. Since there might be multi contamination taken as a single positive result. Nevertheless results from previous studies showed that there is a higher rate of *Campylobacter* isolation after eviscerations but before washing (Woldemariam *et al.,* 2009). Abattoir floors, platforms and walls on most occasions are contaminated due to microorganisms brought in by animals' hides and feces and also through blood droppings and rupture of viscera (Bell and Hathaway, 1996). These all phenomena hold true for the present establishment, for example entrance gate from the lairage to slaughter hall of animals is very near to clean section where washed carcass hang hence animals were frequently passing from the unclean section to the clean.

Personnel and other workers in the abattoir were not adequately trained and hence they mostly do not follow hygienic standards which invariably contribute to the microbial contamination. It was also observed that the abattoir workers put knives into rectal openings of the animal while de-skinning. Besides, knives were not washed frequently between the operations in the abattoir, and there was no provision of hot water bath for knives. The movement of personnel was not restricted and it had also its own impact on the contamination of floors and platforms. The situation is further aggravated by the ridged surfaces on platforms, uneven surfaces, cracks and crevices on the floors and walls where meat particles and moisture were accumulated resulting in the growth and multiplication of bacteria. Yet washed carcasses after inspection directly loaded on transport vehicles mostly without subjection to cold rooms then to butcher shops, hotels and supermarkets. Therefore, environmental sources of contamination play a major role in rendering the meat unsafe for human consumption.

Various investigations from different parts of the world have strongly indicated the emergence of antimicrobial resistant *Campylobacter* strains. One determining factor for the bottle neck is that antibiotic resistant strains in food samples may serve as a reservoir, thereby allowing micro-organisms to persist and spread in the community. Antibiotic resistance is increasing to some antibiotics, such as fluoroquinolones and third-generation cephalosporins. These antibiotics are commonly used to treat serious infections caused by bacterial pathogens frequently found in food, such as *Salmonella* and *Campylobacter* (Bryan and Doyle, 1996).

In this study, drugs under aminoinoglycoside and penicillin family were highly resisted with the examined isolates; amixacillin-clavulanic acid (42.1%), kanamycin (42.1%), sterptomycine (33.3%), oxytetracylin (33.3%) and compound sulphonamide (33.3%). Most of these drugs have been widely used as grows promoters as well as for tackling bacterial infections on animas being in combination with other antimicrobials as a broad spectrum mostly in the injectable forms in Ethiopia.

Fluroquinolone and cephalosporins were the least resisted in the present study that is most of mutton isolates were susceptible to ceftriaxone (4.8%) and clindamycin (4.8%) and to a lesser degree to erythromycin (9.5 %), which are drugs of choice for treatment of acute and complicated cases of the disease (campylobacteriosis) on young children and immunocompromised people. Suggesting these three drugs could continue to be the drugs of choice for treating human campylobacteriosis. Taking advantage on that these drugs are not in use for treatment of food animals in Ethiopia. Previous investigations in Egypt on human and broiler chicken reported erythromycin 62.5% and 58.8% resistance for *C.jejuni* (Nawal, 2011).

Considering the relative resistance of *Campylobacter* spp., *C. lari* displayed higher resistance percentage 3(100% each) towards a few antimicrobial agents that are nalidixic acid, kanamycine, oxytetracycline and compound sulohonamide followed by *C. coli* 2(33.3% each) towards trimethoprim, streptomycin and penicillin G and *C. jejuni 3*(25%) towards kanmaycine, streptomycine and oxytetracycline. Kassa, *et al*. (2005) also reported higher resistance rate of C. *lari* among few isolates from farm animals that is 100% for nalidixic acid, (50%) for trimethoprim sulfamethaxazole and (33%) for streptomycine, clindamycine and erythromycine .

While the multi antimicrobial resistance was observed on (52.4%) the strains higher percentage shown by *C. jejuni* that is (28.6%) then *C. lari* (14.3%) and *C.coli* (9.5%). Higher multi drug resistance of *C.jejuni* isolates of broiler chicken from Egypt was also reported (64.7%) for ampicillin, streptomycin, chloramphenicol and (58.2%) to erythromycin (Nawal, 2011). Though nalidixic acid susceptibility is suggested in literatures as species identification of *C. jejuni* and *C.lari* (16.7%) C. *jejuni* were showed resistance towards this drug and still there are also similar reports on nalidixic resistance of C. *jejuni* from other parts of the world Barret *et al.* (1988); very recent report form USA (6.2%) resistance rate of C. *jejuni* towards nalidixic acid (USDA, 2014) and for *C. coli* by Yeshimebet *et al*. (2013).

6. CONCLUSION AND RECOMMENDATION

The study demonstrated that the sheep and the mutton could be a potential source of thermophilic *Campylobater* spp. with higher isolation rate for *C. jejuni* which is primary cause of human campylobacteriosis for the public when unhygienic slaughtering operation procedures practiced that inevitably result carcass contamination with the bacteria from animal source and the environment. Adding on that, these bacteria from animal source are shown to be emerging to antimicrobial resistant strains. Underlining, food of animal origin may serve as a reservoir for the resistant bacterial strains, thereby allowing micro-organisms to persist and spread in the community. Whereas majority of mutton isolates were susceptible to ceftriaxone, clindamycin and erythromycin. In conclusion, an abattoir based bacteriological survey with the antimicrobial susceptibility patterns is an important input for basis of risk analysis, management and implementation of HACCP.

Recommendations forwarded based on the above conclusions are:

- Various measures should be put in place to minimize the possibility of fecal material being transferred from the gut or the skin to the carcass during the slaughter process.

- Integrated control strategies of ante mortem control (clean livestock policy), hygiene control during slaughter, implementation of HACCP and regular microbiological testing on the abattoir as well as farms should be implemented.

- According to this study, for mutton originated campylobacteriosis ceftriaxone, clindamycin and erythromycin are drugs of choices

- Controlled and careful use of antimicrobials, both in veterinary and human treatment regimens and further wider investigation of antimicrobial resistance pattern for well-targeted use of antimicrobials.

- Intensive education, training and awareness creation for producers, retailers, and consumers on the proper handling and cooking of food of animal origin.

- Further molecular characterization of isolates from animal origin with that of the human and association with epidemiological and demographic factors.

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8. ANNEXES

Annex 1: Preparation of Modified Charcoal Cefoperazone Desoxycholate Agar blood free medium (mCCDA)

Campylobacter Blood-Free Selective Agar Base modified (CCDA-Preston). It was prepared according to the manufacturer's instruction (Oxoid CM 739). 22.75gm of Campylobacter blood free selective agar base was suspended in 500 ml of distilled water and brought to boiling point to dissolve the solids. It was then sterilised by autoclaving at 121'C for 15 minutes. The medium was cooled to 50'C. One vial of antibiotic supplement was added aseptically. This step inhibits the growth of bacteria except *Campylobacter*. CCDA selective supplement (SR 155) was reconstituted with 2ml of sterilized water. This was mixed well and poured into sterile Petri dishes.

Annex figure 1: mCCDA preparation and the SR 155 supplement.

Annex 2: Brain heart infusion

Brain heart infusion media was prepared according to the manufacturer's instruction (Oxoid CM 225). 37 gm of media were added to 1 litre of distilled water and mixed well and distributed into 2 ml screwed tubes and sterilized by autoclaving at 121 ^{0}c for 15 minutes. Stored cultures were removed from the freezer, subcultured and prepared for further processing.

Annex 3: Hippurate hydrolysis test

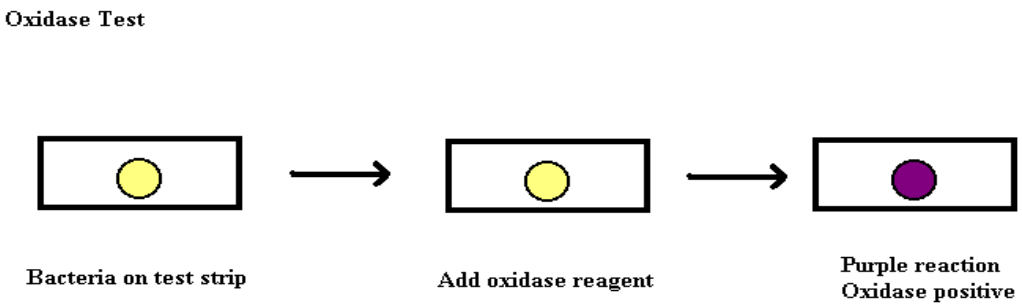
A well-isolated colonies 3-5 from 18-24hr culture was emulsified in demineralized water making a cloudy suspension and by using sterilized tweezers, hippurate disks were dropped in the suspension then incubated aerobically for 2 hrs at 37^{o}c finally 2 drops of ninhydrin reagent added to each test tube re-incubated for 30 minutes at 37oc, observed for blue-purple color development.

Ninhydrin preparation: mix 50ml of acetone and 50ml of 1-butanol thoroughly in dark bottle and add 3.5 gm ninhydrin mix (can be stored at room temperature up to 6 months).

Annex figure: 2 Hippurate test results.

Annex 4: Oxidase test

Method A few crystals of NNN'N'-tetramethyle-p-phenylene-diamine dihydrochloride (Sigma-Aldrich Co. Fancy Road, Poole, Dorset, BH 12 4QH, England) were dissolved in 5 ml sterile deionized water. A sterile cotton swab was soaked into prepared oxidase reagent and was used to select a single colony of the test organism. The appearance of a pink /violet colour within 10 seconds was taken as indicative of a positive reaction.



Source: //www. fouzi.com//

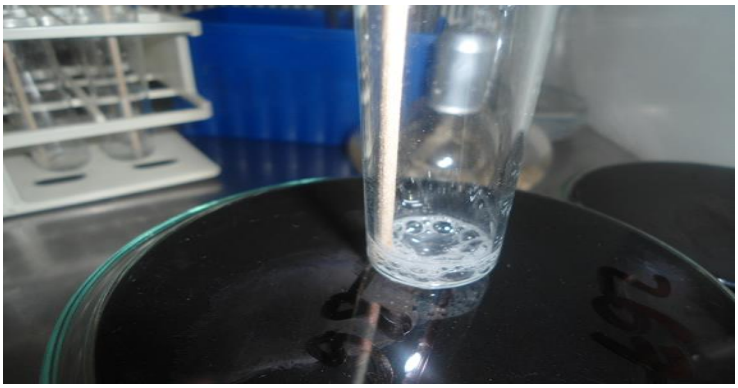
Annex 5: Catalase test

Procedure to carry out Catalase test:

- Tube or bottle method
    - Place 0.2 mL of hydrogen peroxide solution in a test tube
    - Pick a colony to be tested with straight loop
    - Rub the colony onto the inside wall of the bottle above the surface of the hydrogen peroxide solution.
    - Cap the tube and tilt it to allow the hydrogen peroxide solution to cover the colony
    - Look for vigorous bubbling occurring within 10 seconds

Positive result: Vigorous bubbling indicates the presence of catalyse

Negative result: No bubbling



Annex figure 3: Catalase positive reaction with H_2O_2

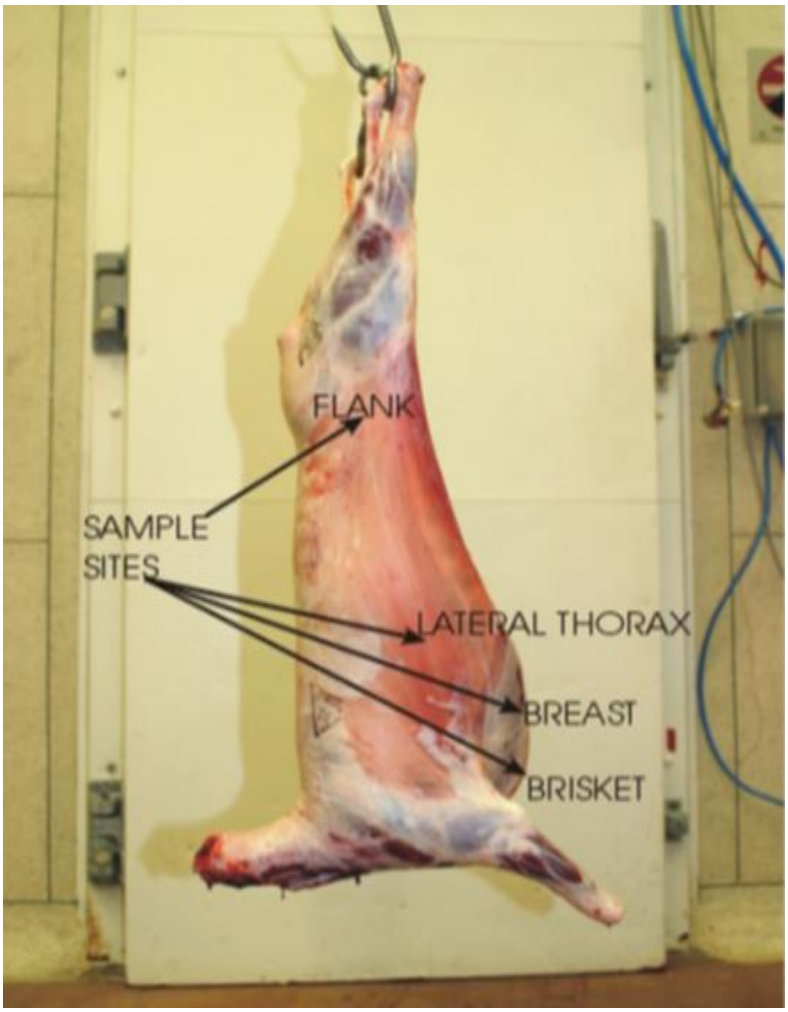
Annex 6: Blood agar base (oxoid blood agar base)

Typical formula (in g1 -1): Protease peptone 15.0; Liver digest 2.5; Yeast extract 5.0; sodium chloride 5.0; Agar 12.0; Ph 7.4 ± 0.2 (CM271).

Medium was prepared according to manufacturer's instruction (Oxoid Ltd, Wade Road, Basingstoke, Hampshire, RG 24 8PW, UK).

Method: The powder was suspended 40 g in I litre of distilled water. The suspension was brought to the boil so all solids were dissolved evenly. The medium was sterilized by autoclaving at 121^0C for 15 minutes for blood agar, the base was cooled to 50℃ and then 7% (v/v) of defibrinated horse blood SR was added. This was mixed with gentle rotation and poured into Petri dishes.

Annex 7: Recommended site for sheep carcass swab sampling.



Annex figure 4: Recommended site for sheep carcass swab sampling

Source: //www.teagasc.ie/publications/2008/1036/Wet_Dry_swabbing.pdf//

Annex 8: Biochemical tests of thermophilic and non thermophilic *Campylobacter* spp.

Characteristic	*C. jejuni*	*C. jejuni subsp. doylei*	*C. coli*	*C. lari*	C. fetus subsp. fetus	*C. hyo-intestinalis*	*"C. upsaliensis"*(b)
Growth at 25°C	-	±	-	-	+	D	-o
Growth at 35-37°C	+	+	+	+	+	+	+
Growth at 42°C	+	±	+	+	D	+	+
Nitrate reduction	+	-	+	+	-	+	+
3.5% NaCl	-	-	-	-	+	-	-
H2S, lead acetate strip	+	+	+	+	-	+	+
H2S, TSI	-	-	D	-	+	+(c)	-
Catalase	+	+	+	+	+	+	-
Oxdase	+	+	+	+	+	+	+
MacConkey's agar	+	+	+	+	+	+	-
Motility (wet mount)	+	+	+	+	+	+	+
Growth in 1% glycine	+	+	+	+	-	+	+
Glucose utilization	-	-	-	-	-	-	-
Hippurate hydrolysis	+	+	-	-	R	-	-
Resistance to naladixic acid	S(d)	S	S	R	S(e)	R	S
Resistance to cephalothin	R	R	R	R		S	S

[a] Symbols: +, 90% or more of strains are positive; -, 90% or more of strains are negative; D, 11-89% of strains are positive; R, resistant; S, susceptible.

[b] Proposed species name.

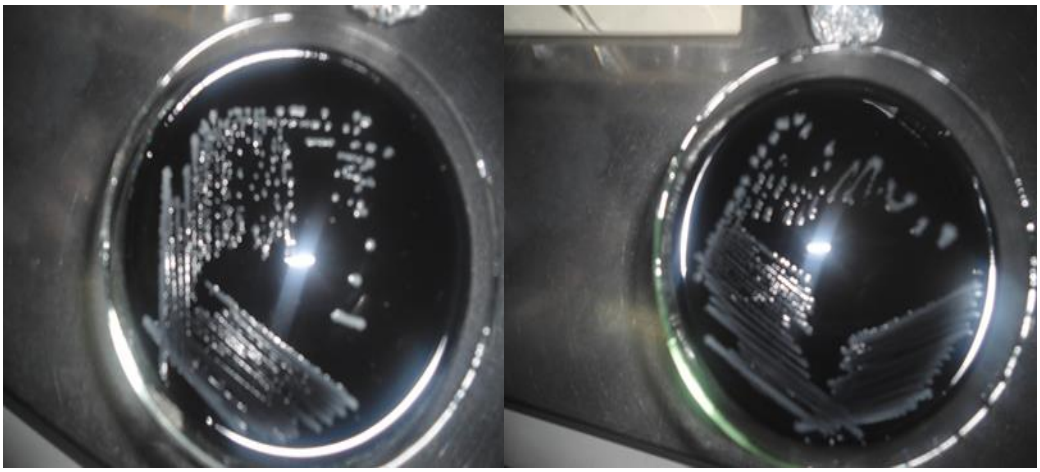
[c] Small amount of H_2S on fresh (<3 days) TSI slants.

[d] Nalidixic acid-resistant *C. jejuni* have been reported.

[e] Cephalothin-resistant C. fetus subsp. *fetu*s strains have been reported.

Source: Barret *et al.* (1988).

Annex 9: Colony morphology of *Campylobacter jejuni on* CCDA



Annex figure 5: *Campylobacter* colony morphology on mCCDA.

Annex 10: LABORATORY FORM

Code no _____

For Laboratory Use Only

The sample collected is of: _____

(I). Carcass (II).Rectal swab (III). Environment (IV). Wash water

Suspected growth of *Campylobacter* spp. from the excreta

A) Positive/present = B) Negative/absent =

Identification steps for suspected colonies

- Motility under microscopy with the iris diaphragm closed__ Y /N
- Gram stain result_____
- Catalase test_____
- Oxidase test_____

- Hydrogen sulfide production _____
- Hippurate hydrolysis test _____
- *Nalidixic acid susceptibility result_____

* results interpreted based on the break points for Enterobacreriacea suggested by CLIS (2012).

Based on reading of the biochemical test, the species of the bacteria is of _____

A) C. *jejuni* B) C. *coli* C) C. *lari*

Antimicrobial susceptibility results (Resistant [R], Intermediate [I], or Sensitive [S])

Ampicillin (AM) 10µg, _____ Amoxycillin-clavulnic acid(AMC) 30µg,___

Ceftriaxone(CRO) 30 µg ____ Penicilline G (P) 10µg, _____

Clindamycin (CM) 2µg, _____ Streptomycin (S) 30µg, _____

Erythromycin (E) 15µg, _____ Kanamycine (K) 30µg, _____

Oxytetracycline (OT) 30µg, ____ Nalidixic-Acid (NA) 30µg, _____

Compound sulphonamide (S_3)300µg. _____ Trimethoprim (W) 5µ_____

Sig._____

Date_____